

Systematics of Sphagnum Frogs of the Genus *Phyloria* (Anura: Myobatrachidae) in Eastern Australia, With the Description of Two New Species

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ABSTRACT. Analyses of allozyme and mitochondrial nucleotide sequence variation in *Phyloria* from northeastern NSW and southeastern Queensland revealed that there are more species than the three that are currently recognized. In addition to the three species presently recognized *P. kundagungan*, *P. loveridgei*, and *P. sphagnicolus*, another two species *P. pughi* n.sp. and *P. richmondensis* n.sp. were recognized under the evolutionary species concept. All species are allopatric. Each of the five species had two or more fixed genetic differences with all other species. Additionally, each species possessed two or more unique allozyme characters (apomorphies). Each species had strongly supported reciprocally monophyletic mitochondrial haplotypes in comparison with each of the other species. Multivariate morphometric analysis was able to distinguish *P. sphagnicolus* from the remaining four northern taxa but was unsuccessful in reliably distinguishing the two new species. Mating call analysis identified two distinct call groups: *P. sphagnicolus* and the remaining species, the latter showing little discrimination between species. The distribution of *Phyloria* in NSW and Queensland shows a strong association with high rainfall rainforest at mid to high altitudes (above 600 m elevation). The habitat of all species is remarkably similar, all are found predominantly in the headwaters of rainforest streams or soaks on the forest floor. All species lay their eggs in nests in the ground, where the larvae remain throughout their entire development until they emerge post metamorphosis. It was confirmed that nests where the embryos were at an early stage of development were of two types: foaming egg masses, and nests containing a non-foaming jelly substance. All species are limited in their distribution. In particular *P. richmondensis* is in need of special conservation consideration, as it is known from only three localities within a very small range. A number of populations in southeastern Queensland and northern NSW await molecular analysis to be identified with certainty.

KNOWLES, ROSS, MICHAEL MAHONY, JAN ARMSTRONG & STEPHEN DONNELLAN, 2004. Systematics of sphagnum frogs of the genus *Phyloria* (Anura: Myobatrachidae) in eastern Australia, with the description of two new species. *Records of the Australian Museum* 56(1): 57–74.

The montane rainforest frogs of the genus *Philoria*, commonly known as “sphagnum frogs” are confined to the Great Dividing Range of eastern Australia (Barker *et al.*, 1995). *Philoria frosti* is the only member of the genus that is not a rainforest species, occurring above the snow line on the Mt Baw Baw plateau in Victoria (Malone, 1985), and it is not dealt with further in the present study. The remaining three members of the genus, *P. kundagungan* (Ingram & Corben, 1975), *P. loveridgei* (Parker, 1940) and *P. sphagnicolus* (Moore, 1958), are found in rainforests from the Mistake Mountains, near Warwick, Queensland, apparently to the Barrington Tops National Park, NSW in the south (Ingram & Corben, 1975; Wotherspoon, 1981). Other than brief habitat and ecological descriptions included in the original species descriptions, only *P. sphagnicolus* has had any aspects of its biology studied in detail (Anstis, 1981; de Bay, 1993; Webb, 1989).

In Australia, 57 of an estimated 194 frog species have been reported in various stages of decline. This represents nearly a third of all Australian frogs (Vial & Saylor, 1993). The montane rainforest species of *Philoria* occur in similar rainforest habitats to several Australian frogs that have disappeared (Mahony, 1996). *Philoria kundagungan* and *P. sphagnicolus* are both found in close association with rainforest streams. Furthermore, populations of their nearest evolutionary relative, *P. frosti*, have recently significantly declined (Hollis, 1995). It is possible that gaining an understanding of the population genetics and ecology of these species of *Philoria* may shed some light on the broader question of the disappearance of eastern Australian frogs.

Philoria kundagungan, *P. loveridgei* and *P. sphagnicolus* are listed as “Vulnerable” under the New South Wales *Threatened Species Conservation Act, 1995*, and the first two are listed as “Rare” in the Queensland *Nature Conservation Act, 1994*. Two fauna surveys funded by NSW National Parks and Wildlife Service in 1989 in the Focal Peaks (Smith *et al.*, 1989b) and Mt Warning caldera (Smith *et al.*, 1989a) regions of northern NSW, both concluded that *Philoria* occurring in these areas were among “... the species most in need of monitoring and special consideration”.

Wildlife managers currently consider the conservation of these species as a high priority (NSW NPWS, 1994). They are among the rarest vertebrates in eastern Australia. *Philoria kundagungan* for instance, is known from only eight localities, all within a very small range. All of the species are habitat specialists restricted to high mountains and throughout their ranges they have been subject to ongoing habitat fragmentation by humans. The need to increase our knowledge of all aspects of the biology of *Philoria* is now being highlighted as a result of legislative protection conferred upon frogs. Three species, *Philoria kundagungan*, *P. loveridgei* and *P. sphagnicolus*, are currently listed as “Vulnerable” in the New South Wales *Threatened Species Conservation Act, 1995*, and the first two are listed as “Rare” in the Queensland *Nature Conservation Act, 1992*. No species are listed as nationally endangered under the Commonwealth’s *Environment Protection and Biodiversity Conservation Act, 1999*. A Fauna Impact Statement with regard to *P. sphagnicolus* in a proposed development area has been required already of a local government council on the NSW north coast by the NSW NPWS (Knowles & Mahony, 1993).

Specimens of *Philoria* collected recently during biological surveys conducted by the North East Forests Biodiversity Study, New South Wales National Parks and Wildlife Service (abbreviated NEFBS hereafter) have not readily conformed to previous taxonomic descriptions. Specimens collected from the Gibraltar Range, outside of the previously known ranges of *P. sphagnicolus*, *P. loveridgei* and *P. kundagungan* have been attributed by different workers to all three species (Australian Museum records, 1993; Queensland Museum records, 1993; NEFBS, unpublished). Different workers have also attributed specimens collected from Mt Warning NSW to either *P. sphagnicolus* or *P. loveridgei* (Australian Museum records, 1993; Queensland Museum records, 1993). The lack of precision in the systematic knowledge of the endangered montane rainforest species of *Philoria* is a severe handicap to efforts to conserve and appropriately manage them. An understanding of the taxonomy, conservation genetics and ecology of the species of *Philoria*, is therefore likely to be crucial to any future efforts to conserve them.

We use a combination of allozyme electrophoretic, mitochondrial nucleotide sequence and morphological analyses to establish a robust species-level systematic framework for *Philoria* in New South Wales and Queensland. We also assess the conservation status of each of the identified species and document the habitat characteristics of the species. The application of the techniques of allozyme electrophoresis and mitochondrial DNA sequencing to define species genetically, followed by morphometric analysis using the groupings so defined, has had significant success in resolving species boundaries within several Australasian vertebrate groups (e.g., Donnellan *et al.*, 1999; Mahony *et al.*, 2001).

Materials and methods

Allozyme electrophoresis. Frozen liver samples were available from 81 specimens of *Philoria* from 23 locations. (Fig. 1 and Appendix). Museum registration numbers and collection locality details are given in the Appendix. Samples from type locations were included where available. Allozyme electrophoresis of liver homogenates was conducted on cellulose acetate gels (“Cellogel”, Chemetron) according to the methods of Richardson *et al.* (1986). The proteins and enzyme products of 34 presumed loci were scored. The proteins that were stained, abbreviations used (Murphy *et al.*, 1996), and their Enzyme Commission numbers (International Union of Biochemistry, 1984) are aspartate aminotransferase (AAT, E.C. 2.6.1.1), aconitate hydratase (ACOH, E.C. 4.2.1.3), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), aldehyde dehydrogenase (ALDH, E.C. 1.2.1.5), carbonate dehydratase (CA, E.C. 4.2.1.1), cytosol aminopeptidase (CAP, E.C. 3.4.11.1), enolase (ENO, E.C. 4.2.1.11), esterase (EST, E.C. 3.1.1.1), fructose-biphosphatase (FBP, E.C. 3.1.3.11), fumarate hydratase (FUMH, E.C. 4.2.1.2), glycerol-3-phosphate dehydrogenase (G3PDH, E.C. 1.1.1.8), glucose-phosphate isomerase (GPI, E.C. 5.3.1.9), glutamate dehydrogenase (GTDH, E.C. 1.4.1.3), L-iditol dehydrogenase (IDDH, E.C. 1.1.1.14), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), lactate dehydrogenase (LDH, E.C. 1.1.1.27), lactoyl-glutathione lyase (LGL, E.C. 4.4.1.5), malate dehydrogenase (MDH, E.C. 1.1.1.37), mannose-phosphate isomerase (MPI, E.C. 5.3.1.8),

nucleoside triphosphate adenylate kinase (NTAK, E.C. 2.7.4.10), peptidases (PEP, E.C. 3.4.11 or 13.*), phosphoglycerate mutase (PGAM, E.C. 2.7.5.3), 6-phosphogluconate dehydrogenase (PGDH, E.C. 1.1.1.44), phosphoglycerate kinase (PGK, E.C. 2.7.2.3), phosphoglucomutase (PGM, E.C. 2.7.5.1), superoxide dismutase (SOD, E.C. 1.15.1.1), and triose phosphate isomerase (TPI, E.C. 5.3.1.1).

Alleles were identified by comparison with samples that were repeatedly included on each gel (internal controls) and through critical side-by-side comparisons (line-ups; see Richardson *et al.*, 1986). For analysis of the allozyme data, specimens of a single genetic type (i.e., a sample with all expected genotypes at variable loci under the assumption of Hardy-Weinberg equilibrium for a single interbreeding population) from a single locality were treated as an OTU (Operational Taxonomic Unit). Geographically proximate OTUs that consisted of a single genetic type were pooled. Cavalli-Sforza & Edwards (CSE) chord distances (Cavalli-Sforza & Edwards, 1967) were calculated with BIOSYS (Swofford & Selander, 1989). A phenogram was constructed from the genetic distances with the neighbour-joining algorithm (NJ—Saitou & Nei, 1987) in PHYLIP version 3.5c (Felsenstein, 1993). A maximum parsimony (MP) analysis was also performed using the heuristic search option of PAUP* 4.0d64 (Swofford, 1999). Loci were treated as characters, alleles as character states and multistate characters were treated as “uncertain” following the recommendation of Kornet & Turner (1999). Support for lineages was assessed with split decomposition using SplitsTree version 3.1 (Dress *et al.*, 1996; Huson, 1998)

Mitochondrial DNA analysis. See the Appendix for details of specimens examined. Total cellular DNA was extracted from frozen liver with the salting out procedure of Miller *et al.* (1988) and stored at -20°C . The same procedure was used to extract DNA from museum vouchers with the modification that the tissue, usually liver, was rinsed several times in TE buffer prior to proteinase K digestion to remove any alcohol. For polymerase chain reaction (PCR) amplifications, 50–100 ng of DNA was added to a 50 μl reaction mixture containing 4 mM MgCl_2 , 1X reaction buffer, 0.8 mM dNTPs, 0.4 mM primers, 1 unit of Biotech *Tth* plus DNA polymerase and the remaining volume of dH_2O . The primers used for amplification of *ND4*, Limno2 [5'-TGA CTA CCA AAA GCT CAT GTA GAA GC-3'] and *ND4* [5'-GRA ATG GNG RGA GGG YTT TTC G-3'], were designed by Schauble *et al.* (2000) and Arévalo *et al.* (1994) respectively. Amplifications, carried out on a Corbett FTS-320 Thermal Sequencer, comprised a single cycle of denaturation for 3 min at 94°C , annealing for 45 s at 55°C and extension for 1 min at 72°C , followed by 29 cycles of 94°C for 45 s, 55°C for 45 s and 72°C for 1 min, ending with a single extension step of 72°C for 6 min. PCR products were purified for sequencing using a Bresa-Clean DNA Purification Kit (Bresatec). Both strands were sequenced directly from each PCR product using the original PCR primers. Products were cycle-sequenced on a Corbett FTS-1 Thermal Sequencer using the Applied Biosystems PRISM Ready Reaction DyeDeoxy Terminator Cycle sequencing kit, following the manufacturer's instructions. The sequencing program comprised 25 cycles of 94°C for 30 s, 50°C for 15 s and 60°C for 4 min. Cycle-sequenced product was electrophoresed and viewed on an Applied Biosystems Model 373A Sequencing System.

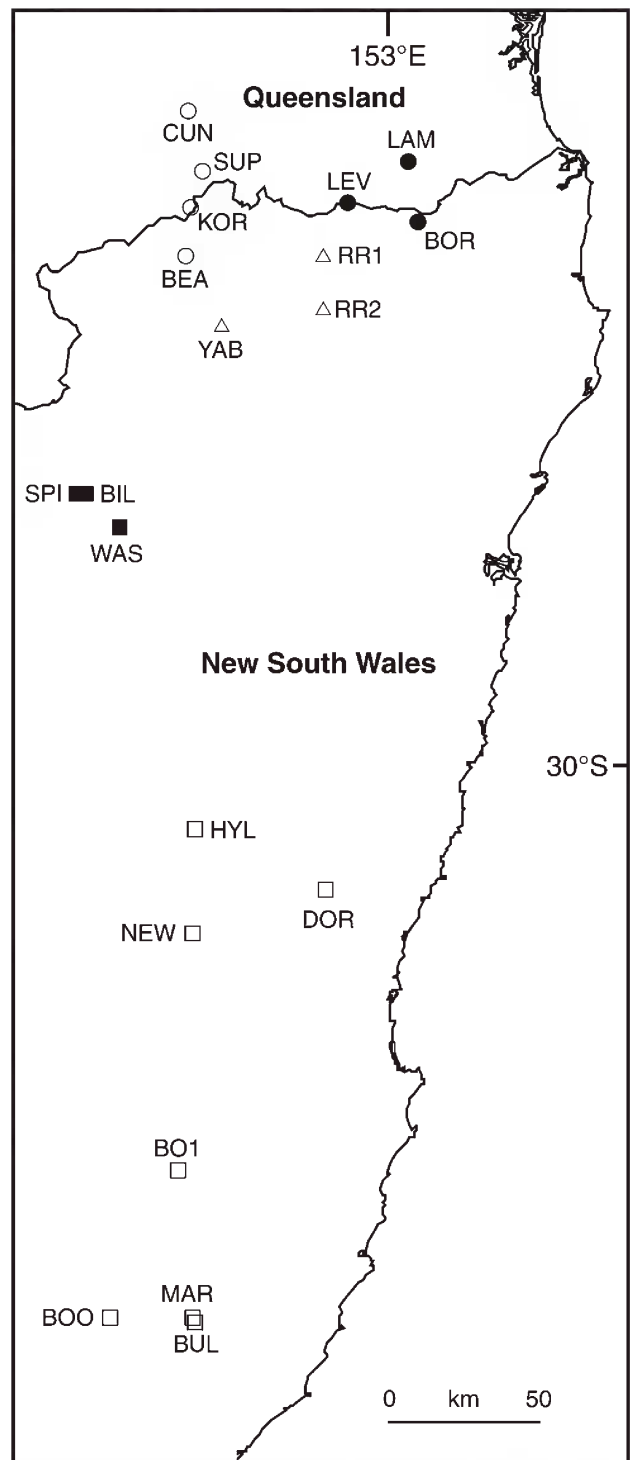


Fig. 1. Map of eastern Australia showing the collection localities for *Philoria*. Abbreviations refer to localities examined for genetic analyses, details of which are listed in the Appendix. Symbols: \circ *P. kundagungan*, \bullet *P. loveridgei*, \blacksquare *P. pughi*, \triangle *P. richmondensis*, \square *P. sphagnicolus*.

A study of relationships within the family Myobatrachidae based on mitochondrial nucleotide sequence data (M. Cunningham, pers. comm.) shows that *P. frosti* is the sister lineage of the species of *Philoria* from northern New South Wales and southeastern Queensland. However, we were unable to amplify *ND4* from *P. frosti*. Instead, a range of limnodynastine genera, *Adelotus brevis*, *Crinia deserticola*

and *Limnodynastes convexiusculus*, sequenced by Schauble *et al.* (2000), were used as outgroups. Sequences were aligned by eye as the alignment did not require the insertion of gaps. Evolutionary trees, constructed with the maximum parsimony (MP), and maximum likelihood (ML) criteria of optimality, were found with heuristic searches implemented in PAUP* 4.0b6 (Swofford, 1999). Modeltest version 3.0 (Posada & Crandell, 1998) was used to assess the most suitable model of nucleotide substitution from the data by hierarchical likelihood ratio tests. Specific model parameters for likelihood analyses were estimated from the data using PAUP* (e.g., base frequencies, transition/transversion ratios, Γ and Γ) by an iterative quartet puzzling method (Leys *et al.*, 2000). The robustness of phylogenetic hypotheses was tested with bootstrapping.

A second set of PCR primers, M372 [5'-GCC GCC GCT CTT CTC TGT AT-3'] and M373 [5'-GGA AGA GAC TAG GCC GTG GG-3'], was designed to amplify a smaller 162bp product from selected museum vouchers. The product, which was sequenced directly, was designed to sample enough diagnostic sites to allow identification of individuals to species.

Morphological analysis. Adult specimens that were allozyme genotyped were included in the morphometric analysis as well as adult specimens held in the Australian and Queensland Museums and the holotypes of each of the described taxa (Appendix). The following characters were measured: snout-vent length (SVL); head width (HW) measured at the widest portion of the skull; head length (HL) measured between the angle of the maxilla and mandible, and the anterior tip of the jaw; tibia length (TL) measured with the knee and ankle joint fully flexed; eye width (E) with the specimen held horizontal; eye to naris distance (EN) from the anterior corner of the eye to the opening of the external naris (nostril) and inter-narial distance (IN) between the external nares. Measurements from formalin-fixed, alcohol-preserved specimens were made with vernier callipers to the nearest 0.5 mm or with an eyepiece graticule on a dissecting microscope. Sex was determined from observation of the presence of a nuptial pad in adults or examination of gonad morphology by dissection.

Colour pattern descriptions are based on live and freshly dead specimens.

Discriminant function analysis (DFA) was conducted with the program Statistica release 5.1, 1997 edition (StatSoft, 1997) to assess differentiation in body shape between the genetic groups. The DFA used the seven variables described above.

Male advertisement call analysis. Analyses were conducted of the male advertisement calls of *Phyllorhina* to determine whether genetically defined groups have diverged in call characteristics. Sound recordings were made of the advertisement calls of male frogs in the field using a Sony WMD6 tape recorder and directional microphone. A minimum of five calls was analysed per male. Call attributes including call (=note) duration, call repetition rate, pulse repetition rate, pulses per call, and dominant frequency range were analysed from audio spectrograms, waveforms and frequency spectra using the sound analysis package Avisoft-SASLab Pro©, 2001.

Larval morphology. The development stage of eggs and larvae were determined under a dissecting microscope according to the criteria of Gosner (1960).

Results

Allozyme electrophoretic data. Table 1 gives the allozyme profiles of the 20 OTUs. For *P. sphagnicolus*, 34 loci were scored, whereas 32 loci were scored for the remaining OTUs of *Phyllorhina*. These data were then converted into matrices of percentage of loci showing fixed allelic differences and CSE chord distances between OTUs.

A NJ phylogram constructed from CSE chord distances between OTUs is presented in Fig. 2A. The BO2 OTU was not included because it appears to be a possible F_1 hybrid (see below), and including a hybrid in an analysis that attempts to produce dichotomous branching trees invalidates an assumption of the algorithm. The OTUs fall into two major genetic clusters: a northern cluster comprising CUN, SUP, KOR, BEA, LAM, BOR, YAB, RR1, RR2, BIL, SPI and WAS and a southern cluster comprising *P. sphagnicolus*—HYL, NEW, DOR, BOO, BUL, MAR, and BO1. The two clusters have fixed differences at a minimum of 28% of their loci. Split decomposition analysis supports the southern cluster. The degree of genetic heterogeneity within both groups suggests the presence of multiple taxa.

Within the northern cluster, four allopatric groups are apparent: group 1 CUN-SUP-KOR-BEA, group 2 LAM-BOR, group 3 YAB-RR1-RR2, and group 4 BIL-SPI-WAS. Group 1 has fixed allelic differences with group 2 at three loci (*Acoh-2*, *Ca* and *Idh-1*), with group 3 at two loci (*Ca* and *Pgk*) and with group 4 at two loci (*Ca* and *Idh-1*). Within group 1, a single fixed difference at *Aldh* distinguishes CUN from BEA. Group 2 has fixed differences with group 3 at two loci (*PepD* and *Pgk*), and with group 4 at one locus (*Acoh-2*). However, LAM has fixed differences with group 4 at four additional loci (*Aat-2*, *Eno*, *Ldh-2*, *Mpi*) and BOR has fixed differences with group 4 at one additional locus (*G3pdh*). The minimum percentage fixed difference between group 2 and group 4 is 6%. Group 3 has fixed differences with the group 4 cluster at a minimum of two loci (*Acoh-2* and *PepD*). Split decomposition analysis supports groups 2 and 3.

The southern cluster comprises two genetic subgroups HYL-NEW-DOR-BO1 and BUL-BOO-MAR. BO2 is a single specimen, which is a probable F_1 hybrid between these two groups. The HYL-NEW-DOR-BO1 subgroup has one fixed difference in allopatry (*Aat-2*) with the BUL-BOO-MAR subgroup to the south. OTUs HYL and DOR, have an additional fixed difference (*Eno*) with BUL-BOO-MAR and OTU BO1 has two additional fixed differences (*Eno* and *G3pdh*) with BUL-BOO-MAR. Overall, there is an average percentage 5% fixed differences between HYL-NEW-DOR-BO1 and BUL-BOO-MAR.

To investigate the nature of possible interaction between the northern and southern subgroups within group 5, we carried out detailed collections in the Hastings River catchment from Mt Boss State Forest (SF) and three locations in Werrikimbe National Park (NP). These specimens were typed subsequently for the three informative loci, *Aat-2*, *Eno*, and *G3pdh*. Typing of the Mt Boss SF/Plateau Beech specimens revealed them to be only the northern type; however, specimens from Cobcrofts and Gorge Creeks in Werrikimbe NP were a mixture of genotypes including southern, F_1 hybrids and F_2 /backcross hybrid genotypes (Table 2). At the Gorge Creek location, electromorph frequencies at each of the three loci typed appear to be consistent with expectations under Hardy-Weinberg equilibrium (Table 2).

Table 2. Genotype frequencies at three loci scored among *Philoria* from two locations in the Hastings River, NSW catchment. χ^2 test (one df) of departure from Hardy-Weinberg equilibrium genotype frequencies. All values were not significant at $\alpha = 0.05$.

locus	observed frequency Gorge Creek		χ^2	observed frequency Cobcrofts Creek		χ^2		
	aa	ab		bb	aa		ab	bb
<i>Aat-2</i>	aa 3	ab 11	bb 3	1.45	aa 1	ab 4	bb 1	3.65
<i>Eno</i>	aa 6	ab 7	bb 4	0.99	aa 1	ab 3	bb 2	3.33
<i>G3pdh</i>	aa 4	ab 7	bb 5	0.25	aa 3	ab 2	bb 0	3.80

Hennigian cladistic analysis of the allozyme electrophoretic data, in which group 5 was used as the outgroup to the northern cluster, revealed a number of unique alleles in each of the taxa identified among the northern OTUs of *Philoria*. Group 1 is characterized by unique alleles at *Acon-2^a*, *Ldh-2^a*, *Mdh-2^c*, *Pgam^d*, and *Pgk^a*; group 2 by unique alleles at *Eno^a*, *Fumh^a* and *Sod^a*; group 3 by unique alleles at *Iddh^a*, *Idh-2^c* and *PepD^b*, and group 4 by unique alleles at *Ca^c*, *G3pdh^d* and *Idh-1^c*. MP analysis found 2726 trees of length 170 steps. Bootstrapping with 2000 pseudoreplicates with the “fast heuristic search” option found support for monophyly of group 1 in 72% of pseudoreplicates, for group 3 in 64% of pseudoreplicates and for the northern cluster in 100% of pseudoreplicates. All other nodes received support from less than 50% of pseudoreplicates.

Mitochondrial nucleotide sequences. Specimens representing the geographic range of each group identified in the allozyme analysis were sequenced. Specimens from Levers Plateau, NSW (LEV) that were not available at the time the allozyme analysis was conducted were included in the nucleotide sequence analysis. The aligned *ND4* nucleotide sequences are available from GenBank, accession numbers are AY178866–901. A total of 616 aligned sites were available of which 272 were variable and 215 were parsimony informative. The most suitable model of nucleotide substitution for these data found with Modeltest was the Tamura-Nei model (Tamura & Nei, 1993) with a specified gamma shape parameter (Γ). Values estimated for parameters of this model were: base frequencies A 0.2629, C 0.3326, G 0.1256, T 0.2787; substitution rates A \leftrightarrow C 0.739, A \leftrightarrow G 14.16, A \leftrightarrow T 0.917, C \leftrightarrow G 0.787, C \leftrightarrow T 4.55, G \leftrightarrow T 1.0; $\Gamma = 0.294$.

An ML tree of the 23 haplotypes found among 26 individuals of *Philoria* sequenced is shown in Fig. 2B. In the MP analysis, 78 equally most parsimonious trees were found of length 543 steps. The strict consensus of the MP trees resembled the ML tree in showing the five major groups that were present in the allozyme analysis. Specimens from the LEV OTU clustered within group 2 (the *P. loveridgei* lineage). Monophyly of the haplotypes within four of the five groups is strongly supported by bootstrapping in both MP and ML analyses (bootstrap proportions greater than 70% *sensu* Hillis & Bull [1993]). The BOR-LAM-LEV (group 2) cluster is not strongly supported in any of the analyses but is nevertheless present in each analysis. Relationships among the five groups are not strongly supported, but some patterns are consistent among the analyses. All analyses place group 2 as the sister lineage to the remaining clusters, and groups 1, 3 and 4 are monophyletic. In contrast many relationships within each

of the five groups are well supported (Fig. 2B). Of particular note is the presence of two divergent haplotype lineages within group 2. The deep divergence between LAM and BOR is also reflected by long terminal branches in the CSE NJ tree based on the allozyme data. In summary, the mitochondrial nucleotide sequence analyses are strongly congruent with the allozyme analyses in that both recover five groups that could be considered as distinct evolutionary lineages as they are supported with data from multiple independent genetic markers.

Morphological analysis. DFA was performed to assess whether each of the genetic groups could be distinguished on the basis of their body shape. Separate analyses were conducted for males and females. An initial DFA was conducted on the specimens that had either been identified *a priori* by allozyme electrophoresis or mitochondrial nucleotide sequencing or were from precise locations where other individuals had been identified from the genetic analyses. The latter action rests on the assumption that a single taxon is present at any one location. As only six *a priori* genetically identified specimens of group 2 were available for morphological analysis, we performed a second DFA including specimens from two other locations in the Border Ranges NP and Lamington NP where the genetically identified specimens had been sampled. This provided 10 additional females of *P. loveridgei*. Sample sizes and a *posteriori* percent correct classifications for the second analysis are presented in Table 3.

Table 3. *A posteriori* percent correct classifications of genetically defined groups (grp 1–5) in discriminant function analysis based on seven variables.

<i>a priori</i> group	% correct	DFA group assignment				
		grp 1	grp 2	grp 3	grp 4	grp 5
females only						
1	100	11	—	—	—	—
2	86	—	12	2	—	—
3	75	1	1	6	—	—
4	60	1	—	1	3	—
5	91	—	—	1	—	10
males only						
1	79	11	1	1	1	—
2	100	—	5	—	—	—
3	75	3	—	9	—	—
4	25	4	—	2	2	—
5	99	—	—	1	—	71

In a forward stepwise DFA of males only, 13 of 111 *a priori* classified cases (12%) were misassigned. Group 2 specimens were correctly assigned in 100% of cases. Classifications for groups 1, 3, 4 and 5 were poorer at 79, 75, 25 and 99% correct respectively. Six variables (TL, E, IN, HL, EN, SV) were retained in the model, with TL making the highest contribution to the first canonical variate. In a forward stepwise DFA of females only, 90, 93 and 100% of group 1, 2 and 5 cases respectively were correctly assigned. For groups 3 and 4, 80 and 67% of cases were correctly classified. Seven of the 49 *a priori* classified cases were misassigned. Group 1 specimens were all correctly assigned, while classifications for groups 2, 3, 4 and 5 were

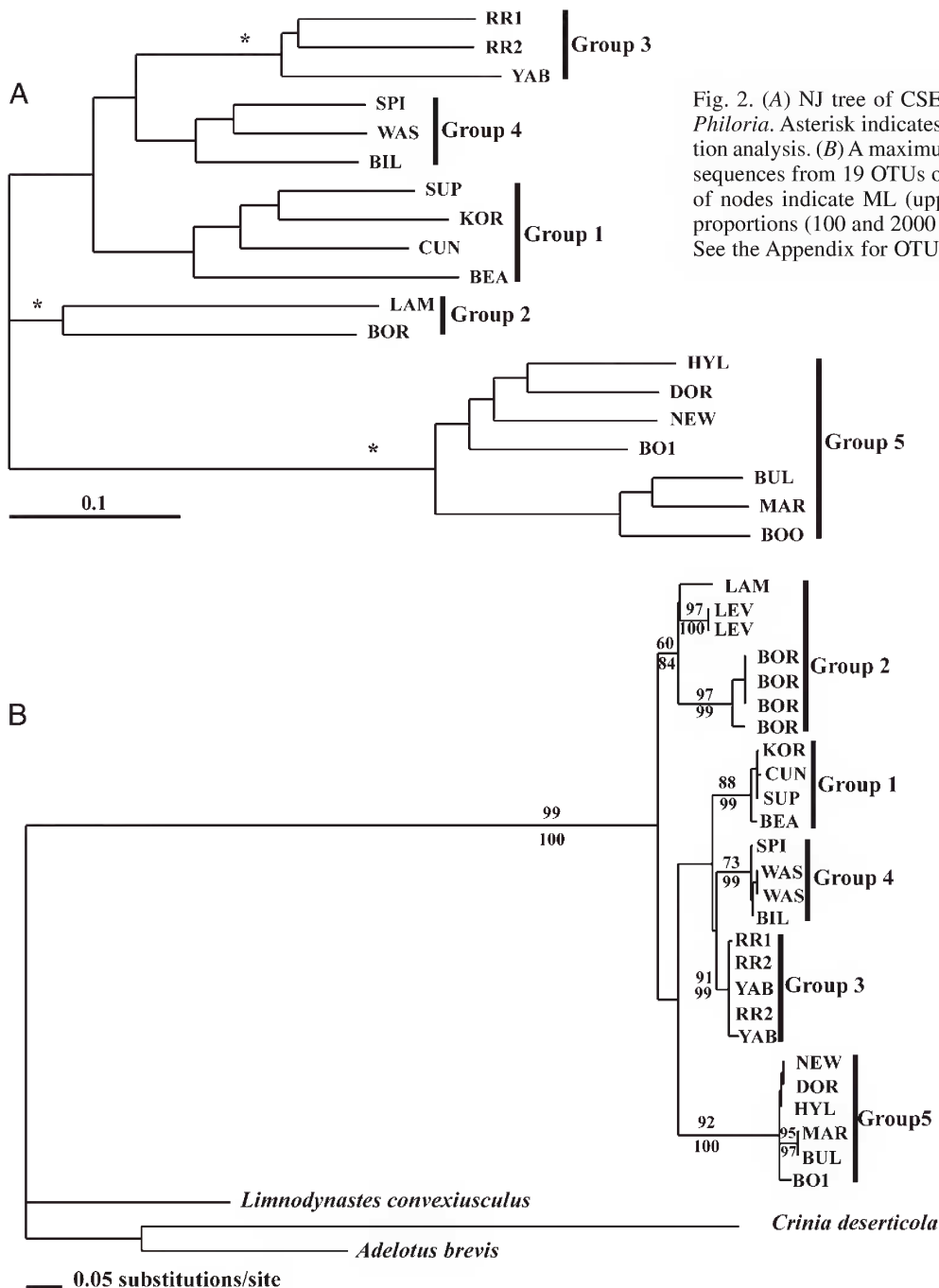


Fig. 2. (A) NJ tree of CSE distances among 19 OTUs of *Philoria*. Asterisk indicates support from split decomposition analysis. (B) A maximum likelihood tree of the 26 *ND4* sequences from 19 OTUs of *Philoria*. Numbers to the left of nodes indicate ML (upper) and MP (lower) bootstrap proportions (100 and 2000 pseudoreplicates respectively). See the Appendix for OTU abbreviations.

86, 75, 60 and 91% correct. Six variables (TL, IN, E, SV, HL, HW) were retained in the model, with TL making the largest contribution to the first canonical variate.

The type specimens of *P. kundagungan*, *P. loveridgei* and *P. sphagnicolus* were entered unassigned to any group. The DFA correctly assigned QM J 23944, the type of *P. kundagungan*, to group 1 with an *a posteriori* probability of 0.61, and AMS R16005 the type of *P. sphagnicolus* to group 5 with an *a posteriori* probability of 0.99. However, BM 1947.2.1994 the type of *P. loveridgei* was assigned to group 3 with an *a posteriori* probability of 0.99, an unexpected finding given that the collection location is within the known range of group 2.

Mating call analysis. The mating calls of five genetic groups of *Philoria* comprise a single note (Table 4, Fig. 3). In most of the groups, the call starts with full energy and tapers off. There is a slight rise to dominant energy in groups 1 and 5.

On the basis of call duration and pulses per call, the genetic groups can be divided into two call types: groups 1 to 4 and group 5 (Table 4). The call of group 5 is approximately four times as long as the other species and the number of pulses per call is between 0.2 and 0.5 times greater and therefore the pulse repetition rate is slower in group 5. The dominant frequencies of the calls are also different, with group 5 having a fundamental frequency at

Table 4. Characteristics of the male advertisement call of the five genetic groups of *Philoria*. n = number of males analysed.

Group—OTU	n	temp (°C)	fundamental and minor component frequency (kHz)	mean call length (sec±SD)	mean call rate	number of pulses per call (range)	mean pulse rate±SD
1—SUP,KOR,BEA	3	11.5–13	0.59	0.1244	7.03	11.2 (10–12)	0.0109
2—BOR	2	15	0.51, 0.23	0.1381±0.025	5.35	9.7 (8–10)	0.0153±0.003
3—SPI	1	10	0.57	0.1232±0.006	6.75	9.5 (8–10)	0.012±0.001
4—RR1	2	na	0.59	0.1672±0.01	5.18	9.5 (9–10)	0.018±0.0003
5—NEW	3	11	0.63, 0.15	0.7301±0.119	8.81	21.5 (14–25)	0.037±0.002
5—DOR	6	12.1–17	0.74, 0.15	0.4945±0.069	8.61	19.3 (11–25)	0.027±0.004

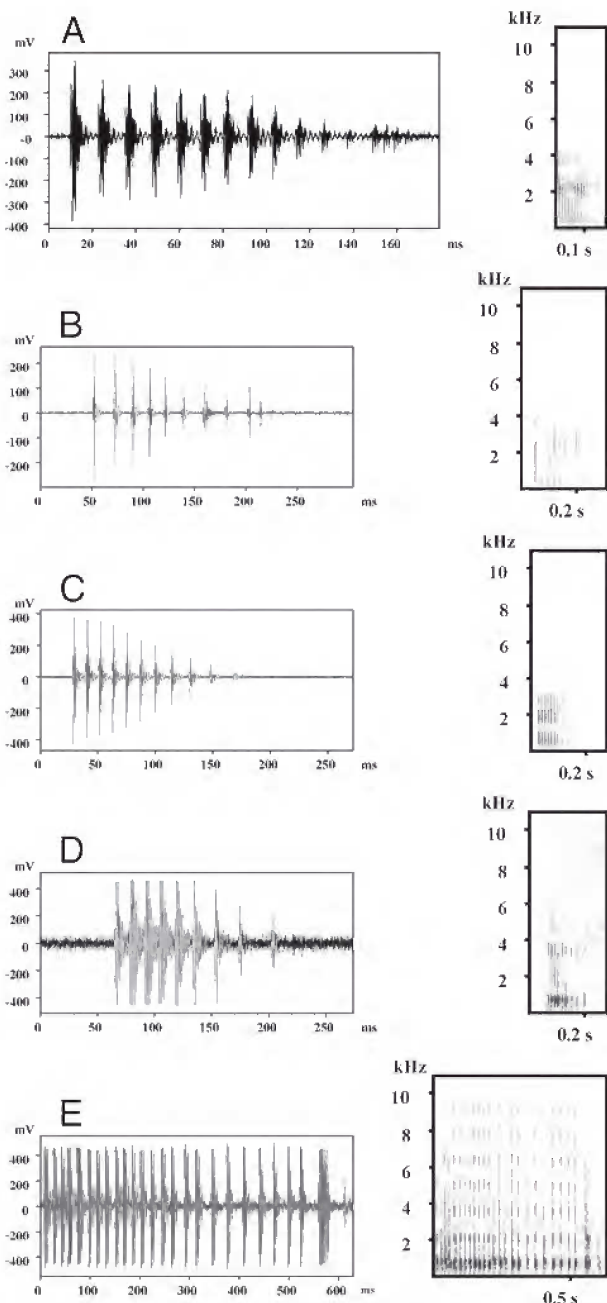


Fig. 3. Waveforms (left) and spectrograms (right) of a single male advertisement call of (A) *Philoria kundagungan* from Mount Superbus (SUP); (B) *P. loveridgei* from Border Ranges NP (BOR); (C) *P. pughi* from Spirabo SF (SPI); (D) *P. richmondensis* from Dome Mountain (RR1); and (E) *P. sphagnicolus* from Calacoma Falls (DOR).

0.9 kHz with a minor component at 1.6 kHz, while the other species have the fundamental frequency at 2.2 kHz, 2.0 kHz, 0.7 kHz, and 0.5 kHz respectively (Table 4).

The calls of groups 1 to 4 are similar in call duration and pulse repetition rate, but they differ in fundamental frequency (Table 4). Groups 3 to 4 have the fundamental frequency of their calls below 1 kHz with minor components at 0.7 and 0.5 kHz respectively, while group 1 has the fundamental frequency at 2.2 kHz, with minor components at 0.4 and 1.2 kHz. Group 1 has a call most similar to group 2 with the fundamental frequency at 2.0 kHz and a minor component at 0.5 kHz in the latter.

Rationale for recognizing species. Virtually any species concept (see Avise [1994] for a recent review) can be used to recognize the presence of phenotypically differentiated sympatric species where there is no hybridization or introgression. However, the basis which we will follow in this paper for the treatment of species boundaries among allopatric populations needs to be discussed. The evolutionary (ESC) and phylogenetic (PSC) species concepts, which rely on the recovery of the historical relationships of the organisms under consideration, have gained recent currency as the basis for the recognition of bisexual species as historical entities among allopatric or hybridising populations. However, the process of induction that allows recognition of species boundaries remains prone to a large degree of subjectivity, as no consensus exists as to how inclusive (PSC) or exclusive (ESC) historical entities should be that we would hypothesize to be lineages i.e., species (Frost *et al.*, 1992). Furthermore, a well resolved “species tree” of recently diverged populations is often not achieved readily. In practice, allopatric populations that can be diagnosed by one or more apomorphic character states irrespective of their perceived potential for hybridization with closely related populations are often accorded specific status (Nixon & Wheeler, 1990; Davis & Nixon, 1992).

In the present study we used phylogenetic analyses of nuclear encoded allozymes and mitochondrial nucleotide sequences to define evolutionary lineages, as apart from comparisons involving *P. sphagnicolus*, none of the male advertisement call data or external morphology show non-overlapping variation. If indeed speciation in *Philoria* has been allopatric resulting from fragmentation of the historical range of the northern *Philoria* (as seems likely), then evidence of post-fragmentation evolution of the populations is required to substantiate an argument for the separate species status. This would be especially acute if the populations were thought to have been fragmented recently and to have been subject to divergence mainly through the

effects of small population size, i.e., accelerated drift. Typically, if a large ancestral population was to be fragmented into smaller disjunct populations, small population size, if maintained for extended periods, would lead to an overall loss of diversity and greater geographic partitioning of the remaining diversity. Our molecular data demonstrate that the taxa have undergone significant evolution in allopatry as each is now reciprocally monophyletic for their mitochondrial genomes and each has a number of apomorphic allozyme alleles consistent with sufficient time having passed for post-isolation mutations to have appeared.

Consequently we present evidence for post-fragmentation divergence. While the type of molecular markers that we have assayed may not measure phenotypic divergence, especially as it pertains to traits that could influence directly reproductive compatibility (Ferguson, 2002), these markers can provide an array of evolutionary measures of genome-wide post-fragmentation divergence and in particular an array of diagnostic loci.

Dealing with the allozyme data first, one would expect that as populations recover from fragmentation (if indeed the scale of fragmentation had been such that the population fragments were small) new allozyme alleles would appear due to mutations. The rate of appearance of new alleles is dependent on the rate of mutation, selection and long-term population size. We note that each of the taxa of the cluster that includes groups 1, 3 and 4 have a number of alleles not found elsewhere in *Phyloria*, i.e. these alleles are lineage-specific autapomorphies. We also note that at the loci that show fixed differences between taxa none of the alleles involved are lineage-specific and therefore their taxon distribution is likely to have been produced by random sorting (and drift) from the putative polymorphic widespread ancestral population.

Each of the taxa is monophyletic for its mitochondrial DNAs. Coalescence theory predicts that reciprocal monophyly for two populations will take $4N_e$ generations, where N_e is the effective population size. So the larger N_e the longer the time taken for reciprocal monophyly to be achieved. There is no *prima facie* evidence that populations of *Phyloria* are small. The current range of each taxon encompasses a number of catchments and population sizes for at least three taxa, groups 1, 2 and 5 must number in the thousands, if not tens of thousands for group 5.

Lastly, mitochondrial genetic distances between taxa are of an order expected for sister species pairs of amphibians. The minimum distance between taxa of the cluster that includes groups 1, 3 and 4 is 5.4% uncorrected sequence divergence, which lies well within the range of values based on the mitochondrial *cytochrome b* gene (2.5% to 19%) for sister species pairs of amphibians surveyed by Johns & Avise (1998). Assuming that rates of change in vertebrate *ND4* are equivalent to that of another protein encoding mitochondrial gene, *cytochrome b* (ie 0.8 to 2.5% per Myr, summarized in Loughheed *et al.*, [1999]), separation of the minimally divergent mtDNA clades occurred some 2.16 to 6.74 Myr ago. These clades have been diverging at least since the early Pleistocene.

Within the cluster that includes groups 1, 3 and 4, populations within each taxon were sampled from a number of catchments and showed very low levels of mitochondrial diversity between samples contrasting with the substantially

greater divergence between taxa. Thus samples from different catchments do not necessarily show high levels of divergence. Similarly, samples from different catchments from within the range of a taxon did not show fixed allozyme differences. These points indicate that genetic cohesion is not confined in its limits to single catchments, i.e. the smallest population units.

Subsequent phenetic analyses of multivariate morphometrics and male advertisement calls supported these lineages in part, i.e. the separation of *P. sphagnicolus* from the remaining taxa. In summary, on the basis of the genetic data, morphology and mating calls, the following species are recognized: group 1—*Phyloria kundagungan* (Ingram & Corben, 1975), group 2—*P. loveridgei* Parker, 1940, group 3—*P. pughi* n.sp., group 4—*P. richmondensis* n.sp. and group 5—*P. sphagnicolus* (Moore, 1958).

Systematics

In addition to describing each of the new taxa, we also redescribe the existing taxa because of the limitations of the identification of *Phyloria* species in New South Wales and Queensland with the presently available literature and the much larger number of specimens available since each of the taxa were described initially. The two new species described below are placed in the genus *Phyloria* in the endemic Australian family Myobatrachidae, based on the diagnoses of Parker (1940) and Cogger (1992) and the genetic data presented herein.

Phyloria loveridgei Parker, 1940

Type data. The holotype is an adult female (BM 1947.2.1994) collected in the McPherson Ranges, Queensland.

The type description lists the collection location of the holotype rather imprecisely as the “McPherson Ranges, 3–4000 ft, south Queensland” (Parker, 1940). Because the McPherson Ranges include the known range of *P. loveridgei* and extends close enough to the known range of *P. richmondensis* n.sp., we cannot be certain that the type was collected within the range of *P. loveridgei* as presently known. Furthermore, the DFA alone is not capable of precisely determining the species identity of any *Phyloria* from far northeast New South Wales and Queensland which includes the McPherson Ranges. Therefore in the interest of maintaining nomenclatural stability we refer the holotype of *P. loveridgei* to group 2, the taxon which contains the populations to which this name has been traditionally applied. These populations include the eastern Border Ranges and Lamington National Parks.

Other material examined. See Appendix.

Diagnosis. Relatively small adult size (SVL to 32 mm); males with poorly developed nuptial pad on first finger; well-developed black head stripe; flanks either entirely black or with a black mark of variable size, and dorsum either brown, reddish-brown bronze or light grey.

Description. A small, squat, ground dwelling frog. Adult males ($n = 15$) measure 25 to 30 mm and females ($n = 13$) 27 to 32 mm SVL. Head shorter than wide (HL/HW mean 0.74, range 0.58–0.91). Head length approximately one-quarter snout-vent length (HL/SVL mean 0.26, range 0.20–0.31). Hind limbs short (TL/SVL mean 0.42, range 0.38–



Fig. 4. Photographs of living specimens of: (A,B) *Philoria kundagungan*—adult males, Koreelah State Forest (KOR); (C,D) *P. pughi*—holotype, an adult male (AMS R152706), and an adult female (ABTC 25369) from Cedar Creek Trail, Washpool NP, NSW (WAS); (E,F) *P. richmondensis*—holotype, an adult male (AMS R152707), and an adult female (ABTC 25225) from Bungdoozle Road, Richmond Range NP, NSW (RR2).

0.47). Eye to naris distance to internarial ratio highly variable (EN/IN mean 0.58, range 0.38–0.69). Dorsal colour varies from light grey mid-dorsal area with black flanks and upper surfaces of limbs, to light brown with darker brown irregular patches to fawn with a few spots of darker pigment and a black or dark brown patch on the flank. Limbs sometimes with faint transverse bands. A dark brown or black stripe extending from the snout, through eye, to base of arm. Ventral surfaces usually uniformly pale but some individuals with light mottling on throat and undersurfaces of hindlimbs. Usually a pair of small dark marks either side of

cloaca. Fingers in decreasing order of length $3 > 2 > 4 > 1$. Toes in decreasing order of length $4 > 3 > 5 > 2 > 1$. In males either no nuptial pad or a dark, very weakly developed nuptial pad on first finger. Spatulae on first and second fingers in females. Vomerine teeth extending to the level of the inner edge of the choanae. Tongue broad. Tympanum indistinct.

Dimensions of holotype (mm). SVL 29.9, HL 7.2, HW 10.5, TL 12.9, E 3.0, EN 1.3, IN 3.4.

Distribution and abundance. Ninety eight specimens are recorded in the Australian and Queensland Museums from 28

locations within one rainforest block which contains the Border Ranges, Lamington, Mt Warning and Nightcap National Parks (Barker *et al.*, 1995; AMS and QM registers, 1993).

Conservation status. Listed under Schedule 2 of the New South Wales *Threatened Species Conservation Act*, 1995 as vulnerable, and in the Queensland *Nature Conservation Act*, 1992 as rare. The reason for the listing in NSW is given as follows: "Population severely reduced; threatening processes severe; ecological specialist" (NSW NPWS, 1994). Whilst the combined area of the range of *P. loveridgei* is relatively small, a comparatively large number of populations are fairly evenly distributed throughout the range. Most of the range is protected within the national park estate. While only small populations were observed during the present study, Seymour *et al.* (1995) observed a number of large populations with choruses of over 10 calling males, in the summer of 1991–1992. Evidence of recruitment was found at all localities during their study. In view of the above, the conservation status of this species remains unchanged.

Phyloria sphagnicolus (Moore, 1958)

Type data. The holotype is an adult male, AMS R16005, collected on 21 November 1952 at Point Lookout, New South Wales.

Other material examined. See Appendix.

Diagnosis. Relatively large adult size (SVL to 37 mm); usually with either an arrow shaped black band across lower back with apex towards head or two black patches on lower dorsum at an oblique angle over the ilium, and males with well-developed nuptial pad on first finger. Male advertisement call has a higher number of pulses per call and a longer call duration than other species of *Phyloria* from NSW and Queensland.

Description. A small, robust, ground dwelling frog. Adult males ($n = 91$) measure 24 to 35 mm and females ($n = 17$) 29 to 37 mm SVL. Head shorter than wide (HL/HW mean 0.68, range 0.57–0.85). Head length approximately one-quarter snout to vent length (HL/SVL mean 0.25, range 0.20–0.30). Hind limbs short (TL/SVL mean 0.46, range 0.38–0.51). Ratio of eye to naris distance to internarial span variable (EN/IN mean 0.63, range 0.44–1.04). Dorsal colour varies from cream through various shades of yellow to orange, red or black usually with irregular spots or patches. Black patches on lower dorsum at an oblique angle over ilium, less frequently extending to and joining at midline in an arrow shape with apex directed anteriorly or, in about one third of specimens, lower dorsum is unmarked. Some dark specimens with a broad cream vertebral band. Dorsal skin usually smooth, occasionally with a few tubercles or short raised ridges along mid-dorsolateral area aligned along long axis of body. Ventral surfaces vary from white to orange often with darker brown to black mottling on abdomen, throat and undersurfaces of limbs. Palms vary from completely dark to completely pale but subarticular and palmar tubercles always pale. Soles completely dark but first and second fingers always pale. A brown or black stripe extending from nostril through eye to base of arm always present but variably developed. Flank with or usually without a black band. A horizontal black band extending laterally from over cloaca to ventral surface of thigh.

Vomerine teeth posterior to and extending to inner edge of choanae. Fingers in decreasing order of length $3 > 2 > 4 > 1$. Toes in decreasing order of length $4 > 3 > 5 > 2 > 1$. Well-developed dark nuptial pad on first finger of males and a spatulae on each of first and second fingers of females.

Dimensions of holotype (mm). SVL 32.5, HL 9.0, HW 11.7, TL 14.4, E 2.8, EN 1.96, IN 3.64.

Distribution and abundance. The distribution of *P. sphagnicolus* extends along the eastern escarpment of the Great Dividing Range in northern NSW from the Marsh SF in the south northwards to Ramornie SF and the vicinity of Glen Innes (Fig. 5). Included in this distribution are four national parks (Werrikimbe, New England, Dorrigo and Bellinger River), two nature reserves (Mt Hyland and Mt Seaview), a number of timber reserves (Mt Banda Banda and Rimau Road) and several state forests (Brooklana, Marengo, Mistake, Mt Boss, Never Never, Nulla Five Day, Oakes, Ramornie, Styx River, and Wild Cattle Creek). The detection of *P. sphagnicolus* in Ramornie SF and just to the east of Glenn Innes represents substantial northern extensions to the range of the species. The Australian and Queensland Museums record 75 specimens from 18 localities, and the NEFBS supplied another location in the Bulga SF. A further 23 locations were found during the course of the present study in the following catchments: Rosewood River, Wild Cattle Creek, Never Never River, Bellinger River, Nymboida River, Forbes River and Hastings River.

The southern-most record of *P. sphagnicolus* with voucher specimens is Marsh SF Wotherspoon (1981) claimed a southern range extension for *P. sphagnicolus* from near the Barrington Tops Guest House, Barrington Tops NP with a report of large numbers of *P. sphagnicolus* foraging on rainforest leaf litter after two days of rain. We doubt the validity of this record for several reasons. Wotherspoon (1981) reported the SVL of one specimen as 52 mm. The largest vouchered specimen of *P. sphagnicolus* reported to date (this study) is 37 mm. Unfortunately, no voucher specimens or photographs were taken and Wotherspoon's is the only record of *P. sphagnicolus* from Barrington Tops NP. Additionally, the site was unlike any other where *P. sphagnicolus* has been found. It was not associated with a stream or running water, which is where large breeding congregations have been found. It is also the only report of *P. sphagnicolus* being observed "foraging on leaf litter". The present authors have never observed *P. sphagnicolus* in the open, but always under some form of cover. During the course of the present study, the site reported in Wotherspoon (1981) was visited after heavy spring rain and large numbers of *Lechriodus fletcheri* were observed moving around the leaf litter and at the edge of small ephemeral ponds on the forest floor. *Lechriodus fletcheri* is reasonably similar in appearance to *P. sphagnicolus* although it is much larger reaching a maximum recorded body length of 50 mm (Moore, 1961; Barker *et al.*, 1995; Cogger, 1992). The 52 mm specimen measured by Wotherspoon (1981) said to be of "average" size for the population, is 2 mm larger than the maximum size of *L. fletcheri* reported in the literature. This discrepancy aside, we consider it likely that *L. fletcheri* was mistaken for *P. sphagnicolus*. However, irrespective of whether *L. fletcheri* was the mistaken species, the evidence outlined above strongly suggests that the population reported by Wotherspoon (1981) was not *P.*

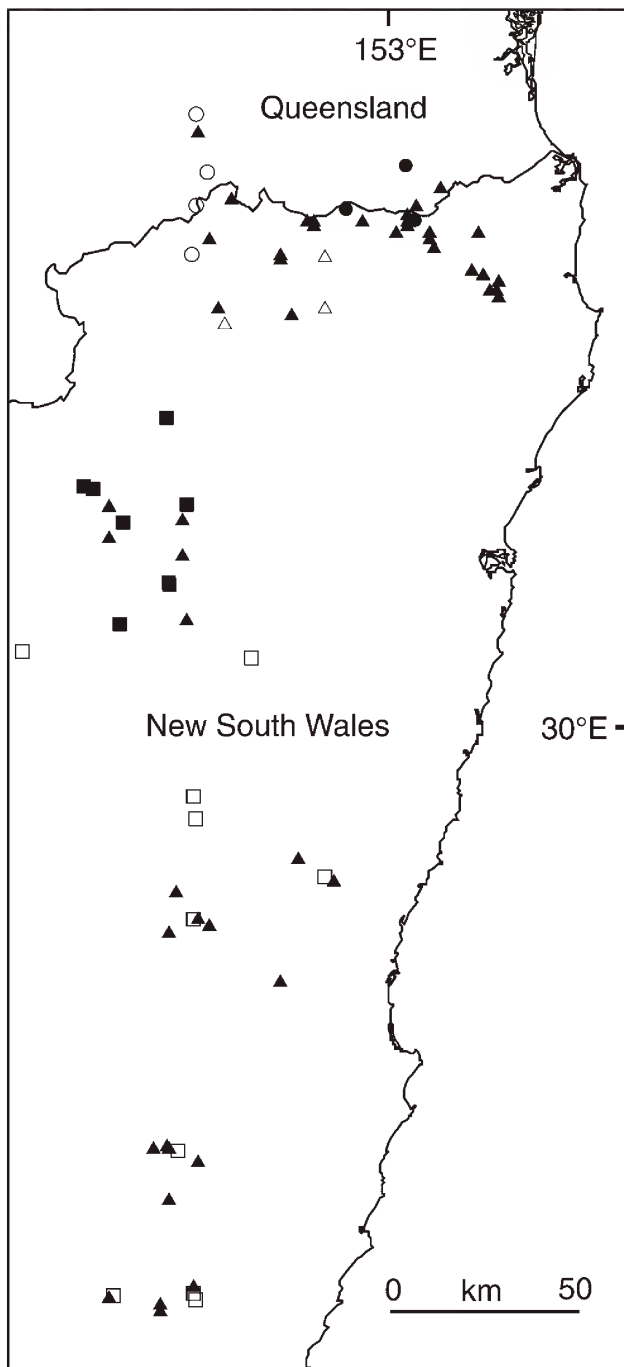


Fig. 5. Map showing distribution of *Philoria* in northern NSW and southeast Queensland based on museum vouchers. Symbols: ○ *P. kundagungan*, ● *P. loveridgei*, ■ *P. pughi*, △ *P. richmondensis*, □ *P. sphagnicolus*, ▲ species' identity not determined.

sphagnicolus. This report is therefore discarded in reviewing the distribution of the species.

Conservation status. Listed under Schedule 2 of the New South Wales *Threatened Species Conservation Act, 1995* as vulnerable. The reason for the listing is as follows: "Population and distribution suspected to be reduced; threatening processes severe; ecological specialist". However, we consider *P. sphagnicolus* to be the most secure of the five taxa studied here for the following reasons:

- As a result of the present study *P. sphagnicolus* is known now from a total of 37 locations, more than double the 18 locations previously available in museum records.
- There appears to be substantial suitable habitat for *P. sphagnicolus* throughout its range. There are a great number of small rainforest streams originating at higher altitudes, and forming part of the four major catchments that drain from the Dorrigo and New England National Parks (Rosewood River, Bellinger River, Never Never River, Wild Cattle Creek). All of these represent potentially suitable habitat for *P. sphagnicolus*. A cause for concern however, is the potential for damage to the habitat of populations in the Wild Cattle Creek catchment through logging operations in Wild Cattle Creek SF. Additionally, development such as clearing for agriculture and forestry has reduced the amount of higher altitude rainforest and this is likely to have decreased the species' abundance. *Philoria sphagnicolus* was observed to be in constant contact with water, hence any water pollution originating from agricultural practices affecting the headwaters of rainforest stream might also impact on local populations.
- There appear to be very few barriers to dispersal of *P. sphagnicolus* throughout its range, the majority of which falls in national parks with extensive wilderness areas. The escarpment between Dorrigo NP and New England NP is very steep and rugged and has a continuous cover of rainforest.
- Breeding congresses observed during the course of the present study were generally relatively small; seven locations had 10 or more calling males in a breeding congress, but two of these had 20 or more. These larger congresses were all observed during November after recent rainfall. Apart from populations of *P. sphagnicolus*, the largest breeding congress observed among the other species was eight calling males of *P. kundagungan* at Mt Superbus, Queensland.

The conservation status of the genetically distinctive southern populations of *P. sphagnicolus* needs further attention. Only six localities are known, four localities are recorded near Elands including the Boorganna Nature Reserve (NR), and one each from Bulga and Marsh State Forests. The largest breeding congress recorded during the course of the present study was at Bulga SF where six calling males were observed. Boorganna NR, the only location that is part of the national park estate, is a very small isolated rainforest pocket surrounded by cleared farmland. The other localities are either on freehold land or in state forests.

Philoria kundagungan (Ingram & Corben, 1975)

Type data. The holotype is an adult female, QM J23944, collected by C.J. Corben and A.K. Smyth on 3 January 1974 at Mistake Mountains, Queensland, 27°53'S 152°21'E.

Other material examined. See Appendix.

Diagnosis. Relatively small adult size (SVL to 28 mm); dark head stripe absent or, if present, narrow; abdomen usually either yellow or red with smaller patches of either colour, and males with poorly developed nuptial pad on first finger (Fig. 4).

Description. A small squat frog with a robust, pear-shaped body. Adult males ($n = 15$) measure 23 to 28 mm and females ($n = 15$) 23.5 to 28 mm SVL. Head shorter than wide (HL/HW mean 0.69, range 0.62–0.79). Head length approximately one-quarter snout to vent length (HL/SVL mean 0.24, range 0.21–0.28). Hind limbs short (TL/SVL mean 0.41, range 0.38–0.46). Ratio of eye to naris distance to internarial span variable (EN/IN mean 0.63, range 0.54–0.71). Dorsal surface varies from either yellow, orange, bright red to black with patches of alternative colours. In about half of specimens, a pair of raised ridges is present on dorsum, starting behind eye and continuing posteriorly about a third of way along dorsum. Dorsal skin either smooth or with raised “warts”, with frequency of “warted” specimens increasing to the south. Ventral surface variably immaculate yellow, yellow with red patches on either throat, abdomen or lateral aspects of abdomen, or yellow with red and brown patches with latter speckled with fine white dots. Undersurfaces of limbs range from yellow to yellow with red or brown wash, latter with small irregular shaped white marks. A black patch over cloaca only or a black patch over cloaca and adjacent upper thighs or entire upper thighs. Fingers and toes unwebbed. Tympanum indistinct. A dark facial stripe is present in about half the specimens running from near tip of snout, through loreal region below canthus rostralis, crossing eye to base of forelimb. Fingers in decreasing order of length $3 > 2 > 4 > 1$. Toes in decreasing order of length $4 > 3 > 5 > 2 > 1$. Nuptial pad on first finger of males weakly developed. Spatulae on first and second fingers of females. Vomerine teeth behind level of choanae.

Dimensions of holotype (mm). SVL 23.5, HL 6.8, HW 8.75, TL 9.35, E 2.7, EN 1.5, IN 1.45.

Distribution and abundance. Found along the ranges from the Mistake Mountains west of Brisbane south to Beaury SF approximately 15 km S of the New South Wales–Queensland border (Ingram & Corben, 1975; AMS and QM registers, 1993). The species is known from 27 specimens from only eight localities. Although Ingram & Corben (1975) make no reference to the abundance of *P. kundagungan*, the small number of known localities and specimens suggest that it is not common. No new locations were found for *P. kundagungan* during the course of the present study.

Conservation status. Listed as rare under the Queensland *Nature Conservation Act 1992*, and under Schedule 2 of the New South Wales *Threatened Species Conservation Act, 1995* as vulnerable. It has a much smaller range than both *P. loveridgei* and *P. sphagnicolus*; it is known from fewer locations, and less specimens have been deposited in museum collections (Ingram & Corben, 1975; Queensland Museum records, 1993; Australian Museum records, 1993). It is known for certain from only eight locations within a very small range. Only three of the known localities, Mistake Mountains NP, Mt Superbus Main Range NP and Cunningshams Gap NP, all in Queensland, are within the national parks estate. All other localities are in state forests. Recruitment was observed at the four locations sampled in the present study. The major threat is likely to be loss or damage to habitat through logging operations. During field work for the present study, logging was observed in the immediate vicinity to *P. kundagungan* habitat at Beaury and Koreelah SF, NSW

Philoria pughi n.sp.

Type data. HOLOTYPE AMS R152706, an adult male from Cedar Creek Trail, Washpool National Park, New South Wales, 29°28'52"S 152°19'05"E (grid reference 4339 67385, zone 56), collected by Ross Knowles on 12 December 1992 (Fig. 4). PARATYPES: SAMA R39818–20 Billilimbra SF, collected by Ross Knowles on 6–8 December 1991; AMS R165008–10 Spirabo SF collected by Ross Knowles on 30 November 1992; AMS R165013 Spirabo SF collected by Ross Knowles on 29 October 1992; SAMA R39241 Spirabo SF collected by Harry Hines on 29 January 1990; AMS R165014 Cedar Creek, Washpool NP collected by Ross Knowles on 12 December 1992.

Other material examined. See Appendix.

Diagnosis. Relatively small adult size (SVL to 30 mm); males with poorly developed nuptial pad; well-developed facial stripe present, and dorsum usually yellow, red or maroon, occasionally light tan or bronze, usually with conspicuous black patch on flank.

Description. Body robust, pear-shaped. Head shorter than wide (HL/HW 0.64), head length approximately one quarter of SVL (HL/SVL 0.23). Snout blunt in profile. Nostrils more lateral than superior, closer to snout than to eye. Distance between eye and naris half that of internarial span (EN/IN 0.58). Canthus rostralis well defined and concave. Eye relatively large, its diameter greater than eye to naris distance (E/EN 1.5). Pupil shape horizontal when constricted. Tympanum small and indistinct. Tongue approximately rectangular. Vomerine teeth in two laterally aligned plates, separated in midline, behind level of choanae.

No webbing on fingers or toes. Digits long, slender and cylindrical. Inner and outer palmar tubercles small but distinct. Fingers in decreasing order of length $3 > 2 > 4 > 1$. Very weakly developed nuptial pad on first finger. Hindlimb short (TL/SVL 0.44). Toes in decreasing order of length $4 > 3 > 5 > 2 > 1$. Inner metatarsal tubercle at base of first toe small but distinct. No outer metatarsal tubercle. Dorsum and abdomen smooth.

Dimensions of holotype (mm). SVL 25.4, HL 5.8, HW 9.1, EN 1.8, IN 3.6, E 2.7, T 11.1.

Colour in life. Dorsum orange fading to yellow on flanks with small flecks of maroon. Crescent shaped black mark on mid-flank. Loreal region dark. A black band expanding posteriorly from snout through nostril along canthus rostralis, through eye along side of head to base of forearm. Blue sclera around eyes. Upper surfaces of limbs maroon, colour extending onto lower abdomen above vent. In preservative, abdomen pale, submandibular area and ventral surfaces of limbs, soles and palms, outer fingers and toes dark. Other fingers and toes with light banding.

Variation. Adult males ($n = 6$) measure 25 to 30 mm and females ($n = 7$) 27 to 30 mm SVL. Head shorter than wide (HL/HW mean 0.72, range 0.65–0.84). Head length approximately one-quarter snout to vent length (HL/SVL mean 0.24, range 0.22–0.30). Hind limbs short (TL/SVL mean 0.42, range 0.38–0.47). Ratio of eye to naris distance to internarial span variable (EN/IN mean 0.63, range 0.55–0.75). Dorsum either smooth or with raised ridges (aligned

along long axis of body) or tubercles along mid-dorsal line and flanks. The description of variation of colour in life is based on colour transparencies of six specimens. Dorsum varies from either plain orange or orange with dark irregular shaped and sized patches and spots or plain maroon. Upper surfaces of limbs maroon. Crescent shaped black mark on mid-flank present in a majority of specimens. Black patch over cloaca, sometimes spreading onto thighs. Ventral surfaces yellow with red patches on abdomen, undersurfaces of hindlimbs mostly red or yellow with numerous red patches. Spatulae on first and second fingers of females.

Etymology. Named for Dailan Pugh (North East Forest Alliance), for his contributions to the protection of the habitat of the species.

Distribution. Confirmed records from seven localities in the Gibraltar Range, Girard, Billilimbra, Forestland, and Spirabo State Forests, and Gibraltar Range and Washpool National Parks to the west of Grafton. The NEFBS (1993) recorded this species from four other localities on the basis of morphological appearance and geographic location. However, these records should be treated with caution as our records include populations of *P. sphagnicolus* in this region. The Australian and Queensland Museums hold specimens from a further eight locations in this area: 20 km SE Tenterfield, the Cangai, Gibraltar Range, Spirabo SF and Washpool SF and Washpool NP. These are probably referable to *P. pughii* on the basis of geographic proximity to OTUs assigned by molecular analyses, but definite assignment awaits genetic analysis. An attempt to sequence *ND4* from AMS R132319 from Cangai SF, 40 km W Grafton was unsuccessful.

Conservation status. The distribution of *P. pughii* is much smaller than the previously recognized distributions of either *P. kundagungan* or *P. loveridgei*, to which this species had been attributed in the past. Only the Gibraltar Range NP and Washpool NP populations are protected within the national park estate with records from 7–12 sites and four other possible but unconfirmed sites. The four populations examined during the present study were all very small. The largest number of calling males observed at any one location was six, at Spirabo SF where the species was found in the boggy headwaters of a small creek. At this site the creek bed had been trampled by cattle in the exact area in which the nests of *P. pughii* were found. Whilst no assessment has been carried out of the potential damaging effects of this trampling on the population's breeding area, the eggs of this species are large and relatively fragile, and would be likely to be damaged or destroyed by trampling by stock.

Philoria richmondensis n.sp.

Type data. HOLOTYPE AMS R152707, an adult male from Bungdoodle Road, Richmond Range National Park, New South Wales, 28°39'19"S 152°42'38"E (grid reference 4717 68357, zone 56), collected by Ross Knowles on 28 October 1992 (Fig. 4). PARATYPES: AMS R165031–3, SAMA R57464 Little Haystack Ck, Yabbra SF collected by Ross Knowles on 3 December 1992; AMS R165028–9 Dome Mountain, Richmond Range SF collected by Ross Knowles on 28 Oct. 1992; AMS R165030, SAMA R57463 Bungdoodle Road, Richmond Range SF collected by Ross Knowles on 27 October 1992.

Other material examined. See Appendix.

Diagnosis. Relatively small adult size (SVL to 28 mm); well-developed head stripe present; dorsum usually yellow, red or maroon, occasionally light tan or bronze; usually without conspicuous black patch on flank; usually with black patches on lower dorsum at an oblique angle over the ilium, and males with poorly developed nuptial pad on first finger.

Description. Body robust, pear-shaped. Head long (HL/HW 0.64), head length approximately one quarter SVL (HL/SVL 0.23). In profile snout prominent and blunt. Nostrils more lateral than superior, closer to snout than to eye. Distance between eye and naris just over half that of internarial span (EN/IN 0.58). Canthus rostralis well defined and concave. Eye relatively large, its diameter greater than eye to naris distance (E/EN 1.5). Pupil shape horizontal when constricted. Tympanum small and indistinct. Tongue approximately rectangular. Vomerine teeth in two laterally aligned plates, separated in midline, behind level of choanae.

No webbing on fingers or toes. Digits long, slender and cylindrical. Inner and outer palmar tubercles small but distinct. Fingers in decreasing order of length 3>2>4>1. Nuptial pad on first finger weakly developed. Hindlimb short (TL/SVL 0.44). Toes in decreasing order of length 4>3>5>2>1. Inner metatarsal tubercle at base of first toe small but distinct. No outer metatarsal tubercle. Dorsum and abdomen smooth.

Dimensions of holotype (mm). SVL 25.4, HL 5.8, HW 9.1, EN 1.8, IN 3.6, E 2.7, T 11.1.

Colour in life. Dorsum bronze, with irregular black band across lower back and fainter black blotches on upper dorsum posterior to eyes and either side along spine. Loreal region dark. A black stripe from snout through nostril along canthus rostralis, through eye along side of head to base of forearm. Eyes with blue sclera. Lips slightly darker bronze than dorsum to brown. Black patches on posterior half of flank between arm and leg. Upper surfaces of limbs bronze with black transverse bands. In preservative, abdomen pale, submandibular area and ventral surfaces of limbs, soles and palms, outer fingers and toes dark. Other fingers and toes with light banding. Cloaca and upper thighs uniformly dark.

Variation. Adult males (n = 8) measure 24 to 28 mm and females (n = 5) 24 to 27 mm SVL. Head shorter than wide (HL/HW mean 0.71, range 0.64–0.82). Head length approximately one-fifth snout to vent length (HL/SVL mean 0.24, range 0.22–0.28). Hind limbs short (TL/SVL mean 0.41, range 0.38–0.47). Eye to naris distance to internarial ratio variable (EN/IN mean 0.63, range 0.55–0.72). Dorsum smooth. The description of variation of colour in life is based on colour transparencies of six specimens. Dorsum varies from bronze or brown to plain orange or orange with a few, small, dark marks or with occasional to numerous dark speckles. Most specimens have black patches on lower dorsum at an oblique angle over ilium, less frequently joining at midline to form an arrow shape with apex directed anteriorly. Upper surfaces of limbs bronze or brown. In some specimens arms with faint transverse black bands. Spatulae on first and second fingers of females.

Etymology. Named after the Richmond Range area that contains the entire distribution of the species.

Distribution. Known with certainty from only three locations within one continuous forest block within the

Richmond Range NP west to Yabbra NP The Australian and Queensland Museums hold several specimens from Bungdoozle Road, Richmond Range NP, Yabbra NP and Toonumbah SF that are likely to be examples of *P. richmondensis* on the basis of their geographic proximity to localities with genotyped specimens. The Australian and Queensland Museums also hold specimens from a number of localities that are geographically intermediate between the distributions of *P. kundagungan*, *P. loveridgei* and *P. richmondensis*. Assignment of these populations awaits genetic analysis.

Conservation status. *Philoria richmondensis* has a very small range and is known definitely from only three localities and possibly another three. The largest number of calling males observed (three) at any locality was at Dome Mountain, Richmond Range NP At both Bungdoozle Road, Richmond Range NP and Yabbra NP, no calling males were observed, but animals were located by turning leaf litter, logs and rocks. These searches were carried out in the months of October and December on field trips where breeding choruses of *P. kundagungan*, *P. pughi*, and *P. sphagnicolus* were observed at other locations. However, evidence of recruitment was found at both Dome Mountain, Richmond Range NP and Yabbra NP, in the form of nests with either eggs or tadpoles. It is likely that clearing for agriculture and forestry in the area surrounding its current distribution has substantially reduced the range of *P. richmondensis*. Urgent attention is needed to assess more fully its conservation status.

Habitat and reproductive biology of *Philoria*

The habitat and reproductive biology of all five species of *Philoria* are so similar that we can describe these features in general and note any exceptions.

Habitat. The potential range of *Philoria* in northeastern New South Wales and southeastern Queensland has now been surveyed extensively, in particular by the NEFBS in the early 1990s. *Philoria* species are distributed along the top of the eastern escarpment of the Great Dividing Range where cool temperate or sub-tropical rainforest occurs above 550 m elevation. Distinct gaps in the distribution can be seen in the drier and warmer Macleay Gorges region, southeast of Armidale, and the Guy Fawkes and Mann wilderness area, southwest of Grafton.

The habitat of all six species is similar (Ingram & Corben, 1975; Barker *et al.*, 1995; Anstis, 1981; Webb, 1989). All species are found predominantly in the boggy headwaters of rainforest streams and around soaks on the rainforest floor. Breeding pairs of all species mate in well-covered sites such as in small chambers excavated in mud and under rock or leaf litter, under the banks of small creeks, or in the sphagnum mat of bogs. Some populations of *P. sphagnicolus* also utilize cracks in rock faces for their nests (Moore, 1958). The predominant feature of all breeding habitats is the saturated state of the substrate.

Reproductive biology. *Philoria kundagungan* breeds from late August to mid February when males call from water-filled cavities under rocks or leaf litter. The eggs of 3.1 mm in diameter are contained in jelly capsules of 4.9 mm

diameter. Eggs are laid directly in a foam nest in water-filled cavities (Ingram & Corben, 1975). *Philoria loveridgei* breeds from November to January, with males constructing smooth-walled chambers in the ground, which are moist but contain no free water (Moore, 1961; Ingram & Corben, 1975; Seymour *et al.*, 1995). Males may be found calling from these chambers, which can be very close to one another anywhere on the forest floor, especially along creeks (Ingram & Corben, 1975; Moore, 1961, Seymour *et al.*, 1995). Eggs in their individual jelly capsules are deposited in the chambers in a sticky, liquefied jelly mass. Unlike the other species there is no foam mass in the chamber. The eggs contain enough yolk to nourish the developing individual to the juvenile stage, hence the larvae do not have a truly aquatic stage (Moore, 1961). We recorded calling males and eggs of *P. pughi* from October to December. Males called in small congresses of up to six individuals. Males of *P. richmondensis* were heard calling in October (a single congress of three males), but eggs were found in October and December. *Philoria sphagnicolus* forms breeding congresses closely associated with creeks (Anstis, 1981). Breeding occurs from October to January with males calling during the day from established nest sites. Egg masses are of the foam type with the jelly mass closely packed with eggs (Moore, 1961). Water has been observed trickling through nest sites. Anstis (1981) speculated that *P. sphagnicolus* may exhibit some form of parental care as both males and females are often found beside a nest of eggs.

The reproductive biology of *Philoria* is unusual in that the embryos complete their entire development and metamorphosis within a nest in the ground. The tadpoles do not leave the nest until they emerge as metamorphlings and derive all their nourishment from the yolk that was contained in the egg. While in the nest, the tadpoles move through a jelly substance that is excreted into the nest by the female at the time of egg-laying (Seymour *et al.*, 1995). Herein lies a significant distinction between the nesting behaviour of *P. loveridgei* and the other species. With the exception of *P. loveridgei*, all of the nests that were observed where the eggs were at early stages of development were found to have foaming egg masses (Table 5). The foam is derived from the jelly substance secreted by the females with the eggs and is beaten with large spatulae on her fingers (Anstis, 1981; Seymour *et al.*, 1995). The foam egg mass provides adequate oxygen for the early stage embryos. Over time, the foam loses its bubbles to become a still jelly. Foam nests were observed up to Gosner's Stage 22–24. All nests with more advanced tadpole stages contained still jelly. *Philoria loveridgei* was observed to have a still jelly nest even when the embryos were in a very early stage of development (Table 5). Seymour *et al.* (1995) found that embryos at the bottom of nests of *P. loveridgei* were deprived of oxygen and eventually died. However, in subsequent stages of development, tadpoles periodically moved to the surface of the jelly and lashed their tails to produce an oxygen rich jelly around them.

Anstis (2002) indicates that the tadpole of *P. loveridgei* (Gosner stage 35) has substantially reduced tail fins and increased tail venation in comparison with *P. sphagnicolus* (Gosner stage 36) and *P. kundagungan* (Gosner stage 33). Comparable observations are not available for *P. pughi* and *P. richmondensis*.

Table 5. Field observations of foam status and number of eggs in 35 nests of *Phyloria*. Data for *P. loveridgei* are from Seymour *et al.* (1995).

species	locations	number nests	nest jelly foam	Gosner's Stage	mean number eggs
<i>Phyloria kundagungan</i>	Cunninghams Gap NP	1	jelly	>35	44
	Mt Superbus NP	1	foam	12–14	51
	Beaury SF	2	foam	22–24	32.5
	Koreelah SF	6	foam	15–17	47.5
<i>Phyloria loveridgei</i>	Border Ranges NP	14	jelly	0–40	31.9
<i>Phyloria pughi</i>	Spirabo SF	4	foam	5–7	40.5
	Washpool NP	1	foam	15–17	42
<i>Phyloria richmondensis</i>	Richmond Range NP	1	jelly	34–35	36
	Yabbra SF	2	foam	15–17	50
<i>Phyloria sphagnicolus</i>	Dorrigo NP	2	foam	5–7	37
	Marsh SF	1	jelly	38–40	—

Discussion

Despite the clear genetic discrimination among the four northern species of *Phyloria*, these taxa show overlapping ranges of variation in both colour and pattern and in body shape as expressed by multivariate metric analysis. Their male advertisement calls are also virtually indistinguishable. As an indication of the difficulty in distinguishing the northern *Phyloria* species, neither of the photographs of *P. kundagungan* or *P. loveridgei* in Barker *et al.* (1995), the latest guide to Australian frogs, are consistent with the species descriptions or the identification key that accompany them. At present the best guide for species identification within *Phyloria*, aside from genotyping, is geographic location, assuming of course that none of the taxa occur sympatrically. Unfortunately this approach does not help to resolve the species identity of a number of populations

around the periphery of the range of *P. pughi* and some others that occur between populations of *P. kundagungan*, *P. loveridgei* and *P. richmondensis*. These may be additional populations of the described taxa or they may represent undescribed taxa. Determination of their identity is a matter of some urgency as at least one of these populations is within land proposed for an extension to a mining development.

Within *P. sphagnicolus*, there is evidence of two groups with relatively distinct mitochondrial haplotype lineages and differentiated across most of their geographic range by a single consistent fixed difference. Evidence of hybridization apparently with random mating patterns was found in a series of locations in the middle of the range of *P. sphagnicolus*. At present we lack comparative call data for the southern group and our knowledge of geographic distributions and degree of genetic introgression is too incomplete to allow a full assessment of the two groups' taxonomic status.

Key to the genus *Phyloria*

- 1 Sides and back above vent with numerous tiny tubercles; usually a conspicuous parotid gland; large adult size (to 45 mm) *P. frosti*
- Sides and back above vent smooth; no conspicuous parotid gland; smaller adult size (to 35 mm) 2
- 2 Intermediate adult size (32 to 37 mm); males with well-developed nuptial pad; usually with either an arrow-shaped black band across lower back with apex towards head or two black patches on lower dorsum at an oblique angle over the ilium; found south of 30°29'S *P. sphagnicolus*
- Small adult size (27 to 30 mm); males with poorly developed nuptial pad; lower back without distinct black band or patches on lower dorsum; found north of 29°28'S 3
- 3 Head stripe absent or if present then narrow; abdomen usually either yellow or red with smaller patches of other colour *P. kundagungan*
- Head stripe well developed; abdomen not yellow or red with smaller patches of other colour 4
- 4 Dorsum brown, reddish-brown, bronze or light grey; flanks entirely black or with a black mark of variable size *P. loveridgei*
- Dorsum usually yellow, red or maroon, occasionally light tan or bronze; flanks with a distinct medial black mark or a continuation of the dorsal colouration 5
- 5 Flank usually with conspicuous black patch *P. pughi*
- Flank usually without conspicuous black patch; found only in Richmond Range area *P. richmondensis*

This key may be of limited value in the event of further populations of *Philoria* are discovered in the northern part of their range in NSW and in southeastern Queensland. An accurate identification may only be possible through genetic methods.

ACKNOWLEDGMENTS. We thank H. Barnes, M. Burghout, H. Hines, D. Milledge, J. Pecar, D. Scotts and A. Smith who either provided specimens or assisted in specimen collection; B. Winton for assistance in the laboratory; P. Jordan and T. Bertozzi who completed the nucleotide sequencing; S. Richards who assisted with the call analyses, and museum collection managers B. Clarke, P. Couper and R. Sadlier for providing access to numerous vouchers. This project was supported in part by funds from NSW NPWS Threatened Species Unit, northern zone, through the assistance of N. Sheppard.

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Manuscript received 5 August 2002, revised 21 March 2003 and accepted 23 April 2003.

Associate Editor A.E. Greer.

Appendix

Specimens of *Philoria* examined. Institution codes: *ABTC*, Australian Biological Tissue Collection, South Australian Museum, Adelaide; *AMS*, Australian Museum, Sydney; *QM*, Queensland Museum, Brisbane; *SAMA*, South Australian Museum, Adelaide. OTU codes are in upper case bold; * indicates specimens sequenced for mitochondrial DNA and typed for allozymes; ^m indicates specimens sequenced only for mitochondrial DNA and unmarked specimens were typed only for allozymes. All specimens used in genetic comparisons were examined morphologically.

Philoria kundagungan: **BEA** Kangaroo Ck, Beaury State Forest AMS R165031–5*; **CUN** Cunninghams Gap National Park (NP) AMS R165136–8,9*,40; **KOR** Acacia Plateau, Koreelah State Forest (SF) ABTC25303 [juvenile], AMS R165127*–30, SAMA R39238–40; **SUP** Mt Superbus AMS R165120*–3,5, 165141, ABTC25292–3. *Philoria loveridgei*: **LAM** Lamington NP AMS R165027*; **BOR** Border Ranges NP AMS R133242^m, 138936*,38–39,42*,59*; **LEV** Levers Plateau ABTC26285^{m/7}. *Philoria pughi*: **BIL** Billilimbra SF SAMA R39818,19*,20; **SPI** Spirabo SF ABTC25208, AMS R165008,9*,10–13, ABTC25359 [larva], SAMA R39241; **WAS** Cedar Creek, Washpool NP AMS R152706*, 165014*. *Philoria richmondensis*: **YAB** Little Haystack Ck, Yabba SF AMS R165031–33^m, SAMA R57464*, ABTC25351, 72630–1; **RR1** Dome Mountain, Richmond Range SF AMS R165028–9*; **RR2** Bungdoozle Road, Richmond Range SF AMS R152707*, 165030, SAMA R57463. *Philoria sphagnicolus*: **HYL** Mt Hyland Nature Reserve (NR) AMS R165015, 165063–4, 165075, ABTC25204–6*; **NEW** Weeping Rock, New England NP AMS R165083, 165068*–9, ABTC24918; **DOR** Rosewood River catchment, Dorrigo NP AMS R165065–7*; **BO1/2** Cockerwombbeba Creek, Mt Boss SF AMS R165099–103, 165052–3, 165062, 165079–80*; King Fern Falls, Werrikimbe NP AMS R165019, 165049, 165098, 165110–21, ABTC12757; Cobcrofts Creek, Werrikimbe NP AMS R165104–6, 165018, 165048, 165088; Gorge Creek, Werrikimbe NP AMS R165022–4, 165045/7, 165055–8,

165085–7/89–92; **BUL** Ellenborough River, Bulga SF AMS R165017–9*; **BOO** Mumfords Creek, Boorganna NR AMS R165078; **MAR** Dingo Creek, Marsh SF AMS R165072*/77, 165118.

Outgroup sequences from GenBank: *Adelotus brevis* GenBank ABR268701; *Crinia deserticola* CDE269697; *Limnodynastes convexiusculus* LCO269707.

Additional specimens genotyped for “short” ND4 fragment: (AMS) *Philoria pughi*—Cedar Ck, Gibraltar Range NP R96794–6; Girard SF R137756–7; Forestland SF R139030/6; Gibraltar Range R31694; Billilimbra SF R139510. *Philoria sphagnicolus*—Marengo SF R145663; Ramornie SF R145674.

Morphology only: (AMS) *Philoria kundagungan*—Cunninghams Gap R38193–4; Koreelah SF R133244; Beaury SF R130862. *Philoria loveridgei*—Wiangarie SF R68515–16/20/22–3/26/28, 86326; Border Ranges R131885–6, 131891, 133242. *Philoria pughi*—Washpool SF R96914. *Philoria richmondensis*—Dome Mountain R132432, 132454/7; Yabba SF R130902. *Philoria sphagnicolus*—Dorrigo NRP20497, 32164–7, 66208; Point Lookout R20947; 17 km E Glenn Innes R32177; New England NP R15738–9, 17162, 20499–500, 71313–6; Elands R93690–1; Boorganna NR R93692; Mt Boss SF R79069, 108707–8, 108730–1/7/9, 108759; Marengo SF R145663.